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2′-Linking of Lipids and Other Functions to Uridine through 1,2,3-Triazoles and Membrane Anchoring of the Amphiphilic Products

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Dedicated to Rainer Mahrwald on the occasion of his 60th birthday

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A straightforward synthesis of 2′-functionalized uridines was developed based on a Cu-catalyzed cycloaddition of 2′-azido-2′-deoxyuridine and functionalized alkynes. The functions comprise biochemically important groups such as lipids, a fluorescent marker (Cy5 analogue), pentaacetylglucose, lysine and biotin, and are linked to the 2′-position of uridine by a 1,2,3-triazole ring. A number of NMR spectroscopic investigations revealed that the lipidated 2′-triazolyl-2′-deoxyuridines anchor themselves in the phospholipid membranes without affecting the molecular order in the double layers; the polar moieties – uracil, ribose and triazole – are located in the lipid/water interface of the membrane.

Introduction

Nucleolipids are conjugates of nucleic acids, oligonucleotides, nucleosides or nucleobases with lipids.[1] They have aroused the interest of researchers in the fields of supramolecular chemistry, functionalization of biocompatible phospholipid membranes, triggered vesicle fusion, gene silencing and biotechnology.[1–17] Several points, such as the 5′-, 2′-, and 3′-positions, and the heterocyclic ring of the nucleobases, have been used for linking the lipid group to a nucleic acid or its constituents. For example, lipid moieties have been linked to the 5′-position of thymidines by Cu-catalyzed cycloaddition of fatty alkynes to 5′-azido-5′-dehydrothymidine, leading to amphiphilic nucleolipids; such compounds form hydrogels and have been considered for a number of pharmacological and biochemical applications.[18–20] If the lipid group is to be situated in the interior of oligonucleotides, positions 3′ and 5′ cannot be used as tethering points, i.e. only the 2′-position and the nucleobases can be used as connection points for the lipid group. Linkage to the 2′-position has been achieved by acylation with fatty acids, by carbamate formation, by alkylation, by disulfide formation of 2′-thiohydroxy-2′-deoxyuridine, or by acylation at 2′-amino-2′-deoxyuridine.[21–25] The latter was obtained from 2′-azido-2′-deoxyuridine by Staudinger reduction.[22] Position 2′ of the nucleosides and oligonucleotides has also been used for tethering other functions such as nucleosides, spin labels, fluorescent labels, electrochemical probes, or terminally functionalized spacer groups.[26–28] It was shown that such 2′-functionalized nucleosides can be incorporated into oligonucleotides.[28–30]

Here, we report on the application of readily available 2′-azido-2′-deoxyuridine to Cu-catalyzed 1,3-dipolar cycloadditions with alkynes.[31] Using this approach it was possible to link lipids and other useful groups such as fluorescence labels, monosaccharides, amino acids or biotin to the 2′-position of uridine through a 1,2,3-triazole linker. Because the 1,2,3-triazole system represents a bioisoster of phosphate or amide, the target molecules can be considered to be 2′-phosphate or amide equivalents.[32] The potency of 2′-azido-2′-deoxyuridine in such cycloadditions has not yet been explored. In this context, it has to be mentioned that Cu-catalyzed azide–alkyne cycloaddition reactions have been recently used for several post-synthetic DNA[26,33] and nucleoside modifications.[34,35] Amongst them, 2′-azido-2-deoxyadenosine derivatives have also been used.[35,36] However, lipid groups have not been tethered to the 2′-position of nucleosides in this way. We will describe a method that allows such an attachment and further disclose the results of studies on anchoring of the lipid-containing 2′-triazolyl-2′-deoxyuridines in phospholipid membranes.

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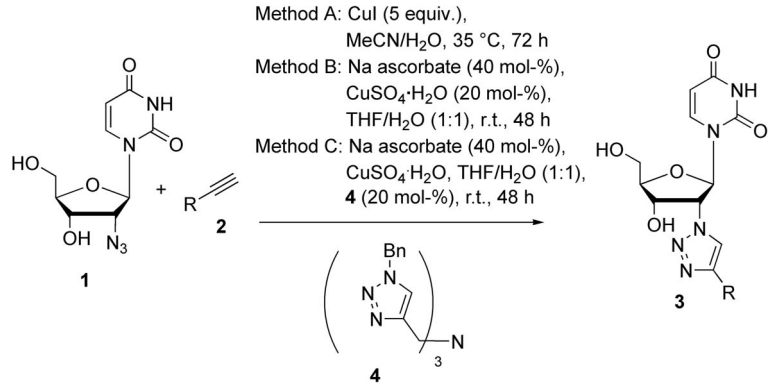


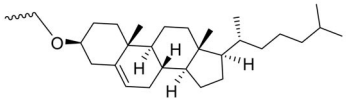
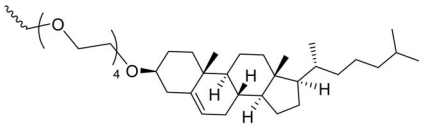
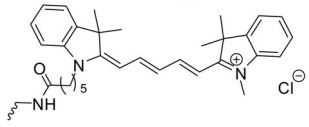
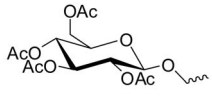
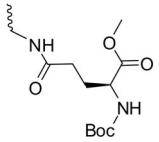
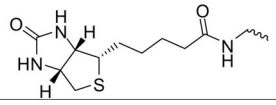
Scheme 1.

Results and Discussion

Synthesis of 2′-Triazolyl-2′-deoxyuridines

Two major procedures for Cu-catalyzed variants of the Huisgen 1,3-dipolar cycloadditions of alkynes to azides are most frequently used: the application of an excess of copper(I) iodide (method A) or of catalytic amounts of copper(II) sulfate in the presence of sodium ascorbate (method B). The former approach (method A) is more flexible in the choice of solvent, whereas the latter is more economical. We applied both methods A and B using acetonitrile and tetrahydrofuran as solvents, respectively (Scheme 1, Table 1). As can be seen in the synthesis of lipidated 2′-triazolyl-2′-deoxyuridines **3a** and **3b**, method B gave better yields. However, there were examples (**2d**, **2f**, and **2g**) wherein method B was unsatisfactory. Thus, method B was modified by the addition of tris(1-benzyl-1,2,3-triazolyl-4-methyl)amine (**4**; method C), which was recently reported to improve the outcome of Cu-catalyzed Huisgen cycloaddition.[37–39] This modification also helped to remarkably improve the yields in our case. However, the overall performance of the method was somewhat hampered by the fact that traces of amine **4** were difficult to remove from the products. Nevertheless, in most cases, high yields could be obtained. Alkynes **2**, which were used as starting materials, are either commercially available or can be easily obtained by introduction of the propargyl group as propargyl alcohol or propargylamine. As shown with one example of each respective case, fluorescence tags (Cy5-analogue, **3d**), carbohydrates (**3e**), amino acids (**3f**) or biological recognition functions (biotin, **3g**) could also be introduced instead of lipid groups in this way. Thus, the Cu-catalyzed addition of alkynes to 2′-azido-2′-deoxyuridine provides a versatile and straightforward access to functionalized uridines, which are interesting for various biochemical and biomedical applications. At present, we are exploring the possibility of incorporating the 2′-triazolyl-2′-deoxyuridines into oligonucleotides and polymers.

Table 1. 2′-Triazolyl-2′-deoxyuridines **3**.

3	R	Yield (%)/Method
a	*n*-$C_{10}H_{21}$	58/A quant./B
b		69/A 83/B 88/C
c		85/B
d		29/B 78/C
e		78/B
f		traces/B 49/C
g		traces/B 57/C

Anchoring in Lipid Membranes

To study the membrane insertion of the two lipidated nucleosides, we used a number of solid-state NMR techniques. We investigated the membrane anchoring properties of molecules **3b** and **3c**, which both carry a cholesterol moiety as a potent membrane anchor, and uridine and triazole as hydrophilic head groups. The two compounds differ only in the length of the polar spacer between the hydrophilic and lipophilic moieties. Such spacers are attractive for biotechnological applications, because they increase the distance between the membrane surface and the DNA oligonucleotide.

We performed standard biophysical NMR experiments to investigate the incorporation of molecules of type **3** into 1-palmitoyl-2-oleoyl-*sn*-*glycero*-3-phosphocholine (POPC)

membranes. To check for alterations in the membrane morphology induced by the presence of the nucleosides, static ^{31}P and ^{2}H NMR experiments were carried out. Figure 1 shows the ^{31}P NMR spectra in the presence of 20 mol-% of each nucleoside.

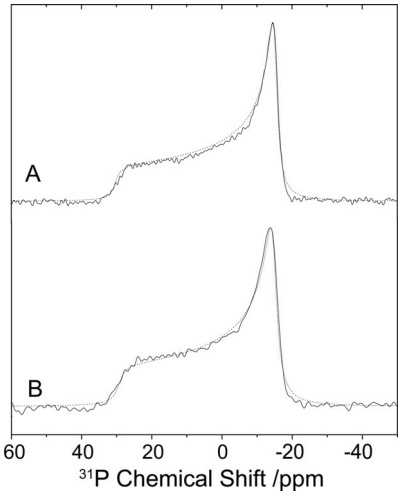


Figure 1. ^{31}P NMR spectra of [D_{31}]POPC in the presence of 20 mol-% **3c** (A) and **3b** (B). The spectra were measured at 30 °C. The samples contained 40 wt.-% H_2O. The dashed lines represent best-fit simulations.

Both spectra exhibit the typical line shape of a lamellar liquid crystalline lipid membrane; there are no indications for the presence of non-lamellar phases. The ^{31}P chemical shift anisotropy (CSA, Δσ), given by the width of the ^{31}P NMR spectra, provides information about the dynamics and orientation of the head group of the POPC molecules.[40] The quantitative values for Δσ (Table 2) are comparable to those of a pure POPC membrane. Only for **3b** was a slightly decreased value obtained. However, considering that the error in the measurement was about ±1 ppm, these small deviations indicate only a very slight variation in the head group dynamics or orientation in the presence of the nucleosides.

Table 2. ^{31}P NMR chemical shift anisotropies, average ^{2}H NMR order parameter, and average chain length of POPC in the presence of 20 mol-% lipophilic nucleotide **3b**, **3c** or 20 mol-% cholesterol for comparison.

Sample	Δσ[a] [ppm]	S_{av}[b]	$<L_c^*>$[c] [Å]
POPC	45.2	0.153	11.46
POPC/3b	44.2	0.155	11.59
POPC/3c	45.5	0.143	11.20
POPC/cholesterol	44.8	0.223	13.33

[a] Chemical shift anisotropy (CSA). [b] Average order parameter. [c] Chain extent according to ref.[44]

To investigate the influence of the nucleosides on the acyl chains of the phospholipids and to study possible lipid condensation effects due to the cholesterol anchor of the nucleotides, ^{2}H NMR spectra of [D_{31}]POPC in the presence of the nucleosides were acquired. The smoothed order parameter profiles obtained from these spectra are shown in Figure 2.

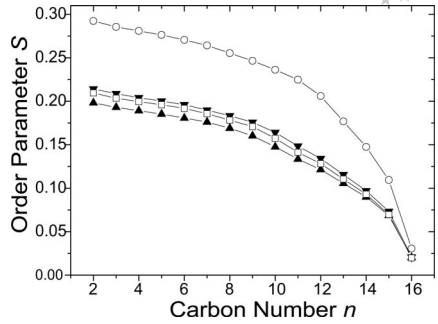


Figure 2. Smoothed ^{2}H NMR order parameter profiles of [D_{31}]-POPC in the presence of 20 mol-% **3c** (▲), **3b** (▼) and cholesterol (○), as well as for pure [D_{31}]POPC (□). The measurements were conducted at 30 °C and with 40 wt.-% H_2O in the samples.

Because both nucleosides exhibit order parameters that are close to those of a pure [D_{31}]POPC membrane – the values are slightly increased for **3b** and slightly decreased for **3c** – there are only minor effects on the lipid chain order due to the addition of the nucleotides to a membrane. The difference between the two nucleotides is most likely caused by the longer spacer of **3c**, which adds additional dynamics and disorder to the membrane. In contrast to cholesterol, neither nucleotide induced any ordering effect. The loss of this condensation effect can be explained by differences in the molecular structures of the nucleosides compared to cholesterol. These modifications alter the very unique cholesterol/phospholipid interactions. Such effects were also observed for cholesterol analogues[41] and other sterols.[42,43] These properties are also reflected in the average order parameter S_{av} and the average chain length $<L_c^*>$ calculated from the order parameters[44] and shown in Table 2.

To provide information about the lipid chain dynamics, square-law-plots are given in Figure 3.

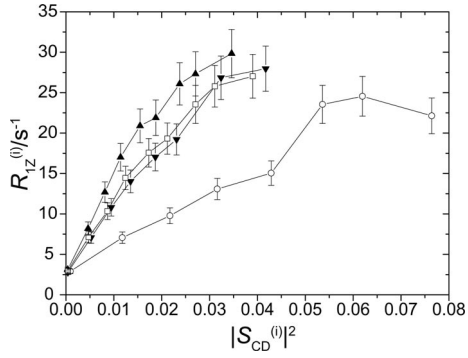


Figure 3. Dependence of the segmental longitudinal relaxation rate $R_{1Z}^{(i)}$ on the corresponding squared order parameter of each methylene segment of [D_{31}]POPC, in the presence of 20 mol-% **3c** (▲), **3b** (▼), and cholesterol (○), as well as for pure [D_{31}]POPC (□). The measurements were conducted at 30 °C. The samples contained 40 wt.-% H_2O.

To this end, the ^{2}H NMR spin-lattice relaxation rates were measured for each methylene/methyl group of [D_{31}]-POPC, and these were correlated to the respective squared order parameter. It is known that the elastic properties of the membrane are mirrored in these plots.[45] For purely sat-

urated phospholipid membranes, these plots exhibit a linear dependence with a specific positive slope.[46] Addition of cholesterol increases the lipid chain order and decreases the slope of the square-law plot, whereas softer membranes (for instance due to the addition of a detergent) exhibit an increased slope and a characteristically curved shape.[45,47,48] The square-law plots of [D_{31}]POPC membranes in the presence of either nucleoside **3b** or **3c** are similar to that of pure [D_{31}]POPC. Both exhibit similar slopes and a small degree of curvature, which is typical for unsaturated phospholipids.[19] None were comparable to the square-law plot of [D_{31}]POPC in the presence of the same molar amount of cholesterol, which again shows that there is no lipid condensation effect induced by the nucleosides. Furthermore, small differences between **3b** and **3c** due to the longer spacer of **3c** are visible, which is consistent with previous observations made from the order parameter plots.

The localization of the lipophilic nucleoside in the membrane was studied by two-dimensional ^{1}H magic angle spinning (MAS) NOESY NMR spectroscopy.[49] For the investigated molecules, this technique allows the distribution of the sugar, the uridine, and the triazole moiety in the membrane to be determined. The cross-relaxation rate, calculated according to the spin-pair model from intermolecular crosspeaks between the moieties of the nucleotides and the segments of POPC, reflect a contact probability between the respective molecular groups. A plot of these values along the membrane *z*-axis enables the distribution of these molecular groups of the nucleosides in the membrane along the normal to be assessed. The lipid coordinates could be taken from a molecular dynamic simulation of POPC,[50] because no significant changes in the membrane due to the addition of the nucleotides were observed.

Because the absolute values of the cross-relaxation rates are influenced by small differences in the correlation times, a normalization of the data was required. The achieved distribution functions along the bilayer normal are shown in Figure 4. The respective molecular groups are rather broadly distributed within the lipid/water interface region of the membrane. Such broad distribution functions are caused by the generally high mobility and disorder in the lipid membrane and have been observed for many different membrane-bound molecules.[42] However, a clear maximum for all molecular segments is visible. For both nucleotides, the distribution functions are very similar for all three molecular groups. For both molecules, the sugar, uridine, and triazole moieties have their highest probability of molecular contacts in the glycerol region of the lipid membrane. Moreover, the contact probabilities in the fatty acid region are significantly lower than for the head group region. Therefore, one has to conclude that the molecular groups of both nucleosides occupy a position in the lipid/water interface of the membrane, which is consistent with the combination of polar character and the possible physical membrane interactions of these molecular groups. Even the long polar spacer in **3c** does not lead to a significant difference in the membrane localization compared to **3b**, which does not carry the spacer.

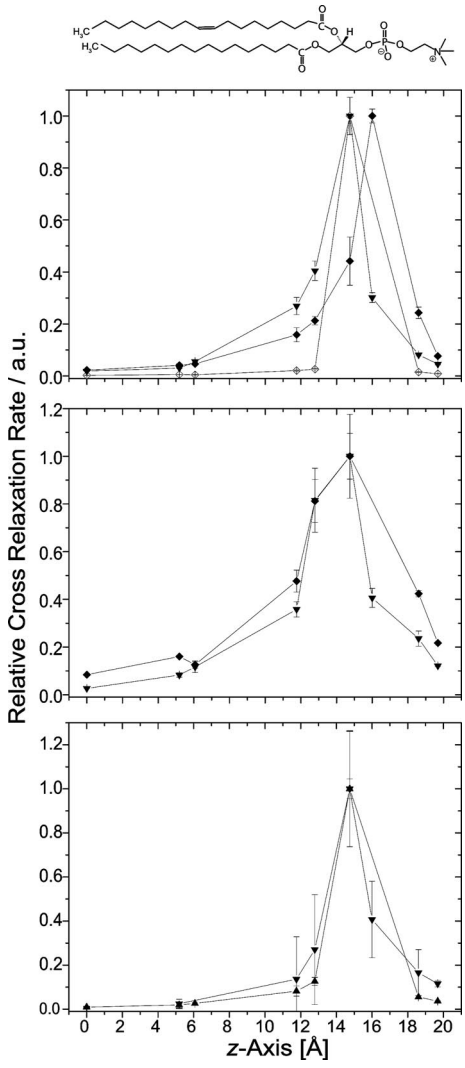


Figure 4. Relative ^{1}H NOESY cross-relaxation rates between the nucleoside moieties of **3c** (♦,◇) and **3b** (▼), and the segments of POPC molecules within the membrane (molar ratio nucleoside/POPC = 1:4) as a function of the coordinates of these lipid segments. The average localization of 6-H and 5-H of the uridine nucleobase (top; for **3c** 6-H ♦, for 5-H ◇; for **3b** the cross peaks of 6-H and 5-H were not resolved so the data set ▼ represents the cross relaxation rates of both protons), of 4-H of the triazole (center) and of the 1′-H of the ribose (bottom), can be estimated. The measurements were conducted at 30 °C. The samples contained 40 wt.-% D_2O.

Furthermore, the small shift in the distribution function of the triazole moiety compared to those of the sugar and uridine moieties should also be noted. This shift reflects the differences in the molecular structure of both nucleotides with the membrane anchor bound to the triazole and the uridine as the polar head group of the molecule.

Conclusions

A straightforward synthetic method has been developed that enables functionalization of the 2′-position of uridine. Starting from 2′-azido-2′-deoxyuridine, a Cu-catalyzed cy-

cloaddition with alkynes was used to link lipid groups, a fluorescent marker, a carbohydrate, an amino acid and biotin as examples of recognition functions to the uridine moiety through a 1,2,3-triazole unit. These products are promising candidates that can be used to build up functionalized oligonucleotides that are interesting for biochemical and medical applications. A range of NMR spectroscopic investigations revealed that the lipidated uridines anchor in biocompatible phospholipid membranes without affecting the order in the double layers, thus demonstrating that the membranes can be equipped with nucleobases through this approach. Furthermore, NMR spectroscopic measurements provided evidence that the uridine moiety and the triazole of the 2′-lipidated uridines are situated in the lipid/water interface of the membrane.

Experimental Section

General Remarks: ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75.5 MHz, respectively, with a Bruker AC 300 spectrometer in $CDCl_3$, with TMS as internal standard. Silica gel (0.04–0.063 mm, Merck) was used for preparative column chromatography. Starting materials **2b**,[51] **2e**,[52] and **2g**[53] were synthesized by introduction of the propargyl moiety according to literature procedures. For the preparation of **2c**, **2d**, and **2f**, see the Supporting Information. All the other materials were purchased from commercial suppliers.

2′-Triazolyl-2′-deoxyuridine 3a

Method A: To a mixture of a solution of 2′-azido-2′-deoxyuridine in MeCN (0.1 M, 4.00 mL, 0.40 mmol) and H_2O (0.40 mL) were added CuI (380 mg, 2.00 mmol) and 1-dodecyne (0.05 mL, 0.40 mmol), and the mixture was stirred at 35 °C for 72 h (TLC showed complete conversion). The solvent was removed, and the residue was purified by column chromatography (CH_2Cl_2/MeOH, 10:1 → 5:1) to give the desired product (100 mg, 0.23 mmol, 57%) as a white foam.

Method B: To a solution of 2′-azido-2′-deoxyuridine in THF (0.5 M, 1.00 mL, 0.50 mmol) were added a solution of $CuSO_4{\cdot}H_2O$ (1 M, 100 μL, 0.10 mmol), a solution of sodium ascorbate in H_2O (1 M, 200 μL, 0.20 mmol) and 1-dodecyne (58 μL, 0.50 mmol), and the mixture was stirred at room temp. for 48 h. The solvent was evaporated, and the residue was purified by column chromatography (CH_2Cl_2/MeOH, 10:1 → 5:1) to give the product (216 mg, 0.49 mmol, quant.) as white foam. R_f = 0.40 (CH_2Cl_2/MeOH, 5:1). M.p. 215–217 °C. ^{1}H NMR ($CDCl_3$): δ = 7.94 (d, J = 8 Hz, 1 H, 6-H), 7.62 (s, 1 H, CH), 6.47 (d, J = 9 Hz, 1 H, 1′-H), 5.66 (d, J = 8 Hz, 1 H, 5-H), 5.25 (m, 1 H, 2′-H), 4.48 (s, 1 H, 3′-H), 4.20 (s, 1 H, 4′-H), 3.72 (m, 2 H, 5′-CH_2), 2.58 (m, 2 H, NCHCH_2), 1.55 (s, 2 H, CH_2), 1.16 (s, 14 H, CH_2), 0.78 (t, J = 6.6 Hz, 3 H, CH_3) ppm. ^{13}C NMR ($CDCl_3$): δ = 163.8, 150.6, 140.4, 102.8, 86.5, 86.1, 70.7, 65.6, 61.1, 31.7, 29.4, 29.3, 29.1, 29.0, 25.3, 22.5, 13.8 ppm. HRMS (ESI): calcd. for $C_{21}H_{34}N_5O_5$ [M + H]$^+$ 436.2549; found 436.2554.

2′-Triazolyl-2′-deoxyuridine 3b

Method A: To a mixture of a solution of 2′-azido-2′-deoxyuridine in MeCN (0.05 M, 8.00 mL, 0.40 mmol) and H_2O (0.80 mL), were added CuI (380 mg, 2.00 mmol) and 2-propargylcholesterol (170 mg, 0.40 mmol), and the mixture was stirred at 35 °C for 72 h (TLC showed complete conversion). The solvent was removed, and the residue was purified by column chromatography (EtOAc/MeOH, 9:1 → 5:1) to give the desired product (180 mg, 0.21 mmol, 69%) as a white foam.

Method B: To a mixture of a solution of 2′-azido-2′-deoxyuridine in THF (0.5 M, 1.00 mL, 0.50 mmol), a solution of $CuSO_4{\cdot}H_2O$ (1 M, 100μL, 0.10 mmol) and a solution of sodium ascorbate in H_2O (1 M, 200 μL, 0.20 mmol) was added 2-propargylcholesterol (210 mg, 0.50 mmol), and the mixture was stirred at room temp. for 48 h. The solvent was evaporated, and the residue was purified by column chromatography (EtOAc/MeOH, 9:1 → 5:1) to give the product (290 mg, 0.41 mmol, 83%) as a white foam.

Method C: A mixture of a solution of 2′-azido-2′-deoxyuridine in THF (0.5 M, 2.00 mL, 1.00 mmol), 2-propargylcholesterol (210 mg, 0.50 mmol) and TBTA (110 mg, 0.20 mmol) was degassed under argon in an ultrasonic bath. Afterwards, a solution of $CuSO_4{\cdot}H_2O$ in H_2O (1 M, 200 μL, 0.20 mmol) and a solution of sodium ascorbate in H_2O (1 M, 400 μL, 0.40 mmol) were added, and the reaction mixture was stirred at room temp. for 1 h. The solvent was evaporated under reduced pressure, and the residue was purified by column chromatography (EtOAc/MeOH, 9:1 → 5:1) to give the desired product (610 mg, 0.88 mmol, 88%) as a white foam. R_f = 0.56 (CH_2Cl_2/MeOH, 5:1). M.p. 283–287 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.83 (d, J = 8.4 Hz, 1 H, 6-H), 7.79 (s, 1 H, CH), 6.35 (d, J = 7.5 Hz, 1 H, 1′-H), 5.48 (d, J = 8.4 Hz, 1 H, 5-H), 5.15 (t, J = 9.6 Hz, 1 H, 2′-H), 5.09 (d, J = 4.2 Hz, 1 H, CH), 4.38 (s, 1 H, 3′-H), 4.02 (s, 1 H, 4′-H), 3.68 (dd, J_1 = 11.7, J_2 = 22.8 Hz, 2 H, 5′-H), 3.07 (s, 2 H, CH_2), 2.00 (m, 1 H, CH), 1.72–0.90 (m, 27 H, CH + CH_2), 0.89 (d, J = 6.6 Hz, 3 H, CH_3), 0.85 (dd, J_1 = 1.2, J_2 = 6.6 Hz, 6 H, CH_3), 0.65 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.8, 150.5, 144.7, 140.3, 121.8, 102.8, 86.5, 85.8, 79.1, 70.7, 65.6, 61.1, 60.8, 42.3, 39.8, 39.5, 39.0, 37.2, 36.8, 36.2, 35.8, 31.9, 28.3, 28.2, 28.0, 24.3, 23.8, 22.6 ppm. HRMS (ESI): calcd. for $C_{39}H_{60}N_5O_6$ [M + H]$^+$ 694.4538; found 694.4470.

2′-Triazolyl-2′-deoxyuridine 3c: To a solution of 2′-azido-2′-deoxyuridine in THF (0.5 M, 1.00 mL, 0.50 mmol) were added an aqueous solution of $CuSO_4{\cdot}H_2O$ (1 M, 100μL, 0.1 mmol), a solution of sodium ascorbate (1 M, 200 μL, 0.20 mmol) in H_2O (1.00 mL) and 11-(5-cholesten-3-yloxy)-1-*O*-(prop-2-ynyl)-3,6,9-trioxaundecane (160 mg, 0.25 mmol), and the mixture was stirred at r.t. for 48 h. The solvent was removed, and the residue was purified by column chromatography (CH_2Cl_2/MeOH, 10:1 → 5:1) to give the desired product (185 mg, 0.21 mmol, 85%) as a white powder. R_f = 0.61 (CH_2Cl_2/MeOH, 5:1). M.p. 183–187 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.07 (s, 1 H, NH), 7.98 (s, 2 H, CH, 6-H), 6.45 (s, 1 H, 1′-H), 5.67 (d, J = 6.0 Hz, 1 H, 5-H), 5.39 (s, 1 H, 2′-H), 5.30 (d, J = 4.8 Hz, 1 H, CH), 5.06 (s, 1 H, 3′-H), 4.64 (m, 1 H, 4′-H), 4.55 (m, 2 H, CH_2O), 3.85 (m, 2 H, 5′-H) 3.59 (m, 16 H, CH_2), 3.14 (m, 1 H, OCH), 2.26 (m, 2 H, CH_2), 2.08–0.90 (m, 29 H, CH, CH_2), 0.89 (d, J = 6.6 Hz, 3 H, CH_3), 0.85 (dd, J_1 = 1.2, J_2 = 6.6 Hz, 6 H, CH_3), 0.63 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.2, 150.6, 144.2, 140.8, 121.7, 79.5, 77.3, 70.7, 70.4, 70.3, 69.6, 67.1, 56.7, 56.1, 50.5, 50.1, 42.3, 39.8, 39.5, 39.0, 37.2, 36.8, 36.2, 35.8, 31.9, 28.3, 28.2, 28.0, 24.3, 23.8, 22.6 ppm. HRMS (ESI): calcd. for $C_{47}H_{74}N_5O_{10}$ [M – H]$^+$ 868.5436; found 868.5413.

2′-Triazolyl-2′-deoxyuridine 3d: To a solution of 2′-azido-2′-deoxyuridine in THF (0.5 M, 0.50 mL, 0.25 mmol) were added an aqueous solution of $CuSO_4{\cdot}H_2O$ (1 M, 50μL, 0.05 mmol), a solution of sodium ascorbate (1 M, 100 μL, 0.10 mmol) in H_2O (1 mL) and 2-(5-{3,3-dimethyl-1-[5-(propagylaminocarbonyl)pentyl]-1,3-dihydroindol-2-ylidene}penta-1,3-dien-1-yl)-1,3,3-trimethyl-3*H*-indolium chloride (110 mg, 0.20 mmol), and the reaction mixture was stirred at room temp. for 48 h. The solvent was removed, and the residue was purified by column chromatography (CH_2Cl_2/MeOH, 10:1 →

5:1) to give the desired product (120 mg, 0.21 mmol, 60%) as a violet foam. R_f = 0.53 (CH_2Cl_2/MeOH, 5:1). M.p. 85–88 °C. ^{1}H NMR (300 MHz, CD_3OD): δ = 7.97 (d, J = 8.1 Hz, 1 H, 6-H), 7.88 (s, 1 H, CH), 7.78 (t, J = 13.5 Hz, 2 H, CH), 7.32 (d, J = 7.5 Hz, 4 H, ArH), 7.18 (m, 2 H, ArH), 7.08 (d, J = 7.5 Hz, 2 H, ArH), 6.49 (m, 2 H, 1′-H, CH), 6.15 (d, J = 13.5 Hz, 2 H, CH), 6.06 (d, J = 13.5 Hz, 2 H, CH), 5.65 (d, J = 8.1 Hz, 1 H, 5-H), 5.29 (t, J = 6.6 Hz, 1 H, 2′-H), 4.52 (m, 1 H, 3′-H), 4.36 (s, 2 H, CH_2O), 4.19 (m, 1 H, 4′-H), 3.92 (m, 2 H, 5′-H), 3.49 (s, 3 H), 2.17 [t, J = 5.1 Hz, 2 H, CH_2(CO)N], 1.80–1.59 (m, 18 H, CH_2, CH_3) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 174.2, 173.0, 172.9, 163.9, 162.1, 153.1, 152.7, 150.6, 142.4, 141.6, 140.8, 140.5, 128.9, 128.7, 128.5, 125.3, 125.2, 125.0, 122.0, 121.9, 111.4, 110.7, 110.3, 107.3, 103.3, 103.0, 102.7, 86.4, 86.3, 80.6, 77.2, 73.1, 70.4, 65.7, 61.0, 35.8, 34.5, 30.8, 29.4, 27.6, 26.8, 26.2, 25.1, 24.8 ppm. HRMS (ESI): calcd. for $C_{44}H_{51}N_8O_6$ [M + H]$^+$ 787.3932; found 787.3926.

2′-Triazolyl-2′-deoxyuridine 3e: To a solution of 2′-azido-2′-deoxyuridine in THF (0.5 M, 1.00 mL, 0.50 mmol) were added an aqueous solution of $CuSO_4 \cdot H_2O$ (1 M, 100μL, 0.1 mmol), a solution of sodium ascorbate in H_2O (1 M, 200 μL, 0.20 mmol) and propargyl-β-D-peracetylglucose (190 mg, 0.50 mmol), and the reaction mixture was stirred at room temp. for 48 h. The solvent was removed, and the residue was purified by column chromatography (CH_2Cl_2/MeOH, 10:1 → 5:1) to give the desired product (255 mg, 0.39 mmol, 78%) as a white foam. R_f = 0.56 (CH_2Cl_2/MeOH, 5:1). M.p. 118–121 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.83 (s, 1 H, NH), 7.99 (s, 1 H, 6-H), 7.95 (s, 1 H, CH), 6.52 (d, J = 7.5 Hz, 1 H, 1′-H), 5.69 (d, J = 6.0 Hz, 1 H, 5-H), 5.35 (m, 1 H, 2′-H), 5.27 (t, J = 9.6 Hz, 1 H, CH), 5.05 (t, J = 9.6 Hz, 1 H, CH), 4.87 (t, J = 9.6 Hz, 1 H, CH), 4.63 (m, 1 H, 3′-H), 4.55 (m, 2 H, CH_2), 4.21 (m, 3 H, 4′-H, 5′-H), 3.75 (m, 2 H, CH_2), 2.04 (s, 3 H, CH_3), 1.97 (s, 6 H, CH_3), 1.84 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.0, 170.7, 169.9, 169.5, 163.6, 150.4, 142.6, 140.3, 125.8, 103.0, 98.4, 86.9, 86.2, 77.3, 72.6, 71.6, 70.9, 70.8, 67.9, 66.0, 65.9, 61.6, 20.6, 20.5, 20.4 ppm. HRMS (ESI): calcd. for $C_{26}H_{32}O_{15}N_5$ [M – H]$^+$ 654.1895; found 654.1894.

2′-Triazolyl-2′-deoxyuridine 3f

Method B: To a solution of 2′-azido-2′-deoxyuridine in THF (0.5 M, 1.00 mL, 0.50 mmol) were added an aqueous solution of $CuSO_4 \cdot H_2O$ (1 M, 100μL, 0.1 mmol), a solution of sodium ascorbate in H_2O (1 M, 200 μL, 0.20 mmol) and (*S*)-N^{α}-Boc-N^{δ}-propargylglutamine methyl ester (150 mg, 0.50 mmol), and the reaction mixture was stirred at room temp. for 48 h. The solvent was removed, and the residue was purified by column chromatography (CH_2Cl_2/MeOH, 10:1 → 5:1). HPLC spectra showed some traces of the desired product, but these could not be isolated.

Method C: A mixture of a solution of 2′-azido-2′-deoxyuridine in THF (0.5 M, 2.00 mL, 1.00 mmol), methyl (*S*)-2-(*tert*-butoxycarbonylamino)-5-oxo-5-(prop-2-ynylamino)pentanoate (230 mg, 0.75 mmol) and TBTA (75 mg, 0.15 mmol) was degassed under argon in an ultrasonic bath. Afterwards, a solution of $CuSO_4 \cdot H_2O$ in H_2O (1 M, 150μL, 0.15 mmol) and a solution of sodium ascorbate in H_2O (1 M, 300 μL, 0.30 mmol) were added, and the reaction mixture was stirred at room temp. for 1 h. The solvent was evaporated under reduced pressure, and the residue was purified by column chromatography (EtOAc/MeOH, 10:1 → 5:1) to give the desired product (210 mg, 0.37 mmol, 49%) as a white foam. R_f = 0.31 (EtOAc/MeOH, 5:1). M.p. 118–121 °C. ^{1}H NMR ($CDCl_3$/CD_3OD): δ = 7.92 (d, J = 8.0 Hz, 1 H, 6-H), 7.79 (s, 1 H, NCH), 6.40 (d, J = 9.0 Hz, 1 H, 1′-H), 5.59 (d, J = 8.0 Hz, 1 H, 5-H), 5.19 (m, 1 H, 2′-H), 4.38 (s, 1 H, 3′-H), 4.24 (m, 2 H, CH_2), 3.72 (m, 2 H, 5′-H), 3.54 (s, 1 H, CH), 2.11 (m, 2 H, CH_2), 1.80 (m, 2 H, CH_2), 1.20 (s, 9 H, CH_3) ppm. ^{13}C NMR ($CDCl_3$/CD_3OD): δ = 172.9, 163.8, 150.5, 140.1, 123.8, 102.7, 86.5, 85.8, 79.8, 70.6, 65.8, 61.0, 52.8, 51.9, 34.4, 31.6, 27.7, 27.2, 27.2 (CH_2), 27.7 (CH_3), 31.6 (CH_2), 34.4 (CH_2), 51.9 (OCH_3), 52.8 (CH_2), 61.0 [CH_2(C5′)], 65.8 [CH(C2′)], 70.6 [CH(C3′)], 79.8 (Cq), 85.8 [CH(C1′)], 86.5 [CH(C4′)], 102.7 (CH-5), 123.8 (CH), 140.1 (CH-6), 150.5 (C-2), 163.8 (C-4), 172.9 (CONH) ppm. HRMS (ESI): calcd. for $C_{23}H_{32}N_7O_{10}$ [M + H]$^+$ 566.2211; found 566.2216.

2′-Triazolyl-2′-deoxyuridine 3g

Method B: To a mixture of a solution of 2′-azido-2′-deoxyuridine in THF (0.5 M, 2.75 mL, 1.37 mmol), a solution of $CuSO_4 \cdot H_2O$ (1 M, 275μL, 0.14 mmol) and an aqueous solution of sodium ascorbate in H_2O (1 M, 400 μL, 0.28 mmol) was added *N*-(prop-2-ynyl)biotinamide (390 mg, 1.37 mmol), and the mixture was stirred at room temp. for 48 h. The solvent was removed, and the residue was crystallized from H_2O to give the desired product (12 mg, 0.02 mmol, 2%) as a white foam.

Method C: A mixture of a solution of 2′-azido-2′-deoxyuridine in THF (0.5 M, 1.30 mL, 0.35 mmol), *N*-(prop-2-ynyl)biotinamide (95 mg, 0.35 mmol) and TBTA (35 mg, 0.07 mmol) was degassed under argon in an ultrasonic bath. Afterwards, a solution of $CuSO_4 \cdot H_2O$ in H_2O (1 M, 68 μL, 0.07 mmol) and a solution of sodium ascorbate in H_2O (1 M, 136 μL, 0.14 mmol) were added, and the reaction mixture was stirred at room temp. for 1 h. The solvent was evaporated under reduced pressure, and the residue was crystallized from MeOH to give the desired product (110 mg, 0.20 mmol, 57%) as a white foam. R_f = 0.01 (EtOAc/MeOH. 5:1). M.p. 262–268 °C (decomp.). ^{1}H NMR ([D_6]DMSO): δ = 11.31 (s, 1 H, NH), 7.92 (d, J = 8.0 Hz, 1 H, 6-H), 7.83 (s, 1 H, NCH), 6.35 (d, J = 9.0 Hz, 1 H, 1′-H), 5.61 (d, J = 8.0 Hz, 1 H, 5-H), 5.25 (m, 1 H, 2′-H), 4.31 (m, 1 H, 3′-H), 4.24 (m, 2 H, CH_2), 4.01 (m, 2 H, 4′-H, CH), 3.57 (m, 2 H, 5′-H), 3.01 (m, 1 H, CH), 2.65 (m, 2 H, CH_2), 2.00 [m, 2 H, CH_2(CO)N], 1.21–1.50 (m, 8 H, CH_2) ppm. ^{13}C NMR ([D_6]DMSO): δ = 172.1, 162.9, 150.4, 140.1, 123.8, 102.3, 85.7, 85.6, 69.1, 64.7, 61.0, 60.9, 59.2, 55.3, 38.2, 30.4, 28.2, 27.9, 25.1 ppm. HRMS (ESI): calcd. for $C_{22}H_{29}N_8O_7{}^{32}S$ [M + H]$^+$ 549.1880; found 549.1895.

Membrane Incorporation of Lipophilic Nucleosides: 1-Palmitoyl-2-oleoyl-*sn-glycero*-3-phosphocholine (POPC), 1-[D_{31}]palmitoyl-2-oleoyl-*sn-glycero*-3-phosphocholine ([D_{31}]POPC) and cholesterol were purchased from Avanti Polar Lipids (Alabaster, AL) and used without further purification. The samples for ^{2}H, ^{31}P and ^{1}H NOESY NMR measurements were prepared as described previously.[19] All samples had a water content of 40 wt.-%. ^{31}P NMR spectra were obtained with a Bruker DRX600 NMR spectrometer (Bruker BioSpin, Rheinstetten, Germany) at a resonance frequency of 242.8 MHz for ^{31}P by using a Hahn-echo pulse sequence with a 90° pulse and a duration of 11 μs, a Hahn-echo delay of 50 μs, a spectral width of 100 kHz, and a recycle delay of 3 s. Continuous-wave proton-decoupling was applied during signal acquisition. Spectral simulations of the ^{31}P NMR line shape were carried out to obtain the chemical shift anisotropy ($\Delta\sigma$) by using a program written in Mathcad 2001 (MathSoft Engineering & Education Inc., Cambridge, MA). ^{2}H NMR spectra were recorded with a Bruker Avance750 NMR spectrometer at a resonance frequency of 115.1 MHz for ^{2}H by using a solids probe with a 5 mm solenoid coil. The ^{2}H NMR spectra were obtained with a quadrupolar echo sequence and a relaxation delay of 1 s. The two 3 μs π/2 pulses were separated by a 60 μs delay. ^{2}H NMR spectra were depaked, and order parameters for each methylene group in the chain were determined as described previously.[54] For the ^{2}H NMR T_{1Z} relaxation studies, a phase-sensitive inversion recovery quadrupolar echo-

pulse sequence with 11 delay times between 1 ms and 2.0 s was used. For the analysis, the line shape of the ^2H NMR powder spectra with the longest delay time was simulated by a superposition of the respective number of Pake doublets by using Mathcad 2001; the program determines the relaxation time for each individual Pake doublet by a fitting procedure that calculates the ^2H NMR spectrum for each inversion recovery delay and compares it with the experimental spectrum. ^1H MAS NMR spectra were acquired at a spinning frequency of 600.1 MHz with a Bruker DRX 600 NMR spectrometer using a 4 mm HR MAS probe. Proton $\pi/2$ pulse lengths were 9.7 μs. A ^2H lock was used for field stability. Two-dimensional ^1H MAS NOESY spectra were acquired at various mixing times (between 1 and 600 ms). The dwell time of the indirect dimension was set equal to one rotor period to avoid folding of spinning sidebands into the central band region of the 2D NOESY spectra. In the indirect dimension, 420 data points were acquired with 32 scans per increment at a relaxation delay of 3.5 s. The volumes of the diagonal and cross-peaks were integrated by using the Bruker Topspin 2.1 software package. NOE build-up curves were fitted to the spin-pair model to obtain cross-relaxation rates.[8] All spectra were acquired at a temperature of 30 °C.

Supporting Information (see footnote on the first page of this article): Synthetic procedures and analytical data of starting materials **2c**, **2d**, and **2f**, and the procedure for the preparation of the lipidated uridine/lipid samples for NMR measurements.

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